## SUPPRESSION OF TISSUE GRAFT REJECTION BY SPERGUALIN

Sir:

Spergualin, (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione[1,2], is an antitumor antibiotic produced by *Bacillus laterosporus*. Spergualin exhibits antitumor effect against mouse leukemia L1210, P388, C1498, mastocytoma P815, lymphoma EL4 *etc.*[3] On the other hand, we found that spergualin had dose-dependent immunosuppressive activities and might be a useful immunosuppressive agent. The results are described in this paper.

As shown in Table 1, spergualin at 20 to 80 $\mu$g/ml suppressed Con A-stimulated blastogenesis and also suppressed LPS-stimulated blastogenesis at 5 to 80 $\mu$g/ml.

Spergualin, daily for 3 days, suppressed the production of antibody to sheep red blood cells (SRBC) in mice. However, spergualin did not inhibit the production of CFU-C (colony forming unit in culture) in bone marrow cell cultures (data not shown).

Testing the effect of spergualin on delayed type hypersensitivity (DTH) to SRBC in mice by a method described in a previous paper[4], it reduced DTH response at doses of 3.13 to 50 mg/kg/day when given daily for three days starting one day after the immunization (Table 2). The administration of 50 mg/kg/day showed almost complete suppression. When spergualin was administered for three days before the immunization, it did not cause any suppression (data not shown). The effect of one injection was much weaker.

The results described above suggested that large doses of spergualin might suppress B and T lymphocyte functions. Furthermore, we confirmed that spergualin promotes transplantation of allogeneic mastocytoma and also skin graft. Cells ($3\times10^7$) of mastocytoma P815 (H-2$^d$) were inoculated into C57BL/6 (H-2$^b$) and the alloreactive cytotoxic T lymphocyte activity of the spleen cells was tested by determining $^{51}$Cr release[5]. As shown in Table 3, spergualin reduced $^{51}$Cr release when 5 or 50 mg/kg was given daily for 9 days from day 1. It was almost completely inhibited by 50 mg/kg/day.

The effect on skin graft was examined by the method described by DENHAM *et al.*[6] except that the Hooded and Wistar rats were replaced with SHR and Fischer (F344) rats. Skin graft was

Table 2. Effect of spergualin on delayed type hypersensitivity to SRBC in mice.

| Spergualin (mg/kg/day) | Footpad thickness increase (mm±SD) |
|---|---|
| 0 | 0.82±0.34 |
| 0.78 | 1.05±0.30 |
| 3.13 | 0.44±0.25* |
| 12.5 | 0.22±0.18* |
| 50.0 | 0.07±0.06* |

Female CDF$_1$ mice of 15 weeks old were used. A group consisted of 8 mice. Mice were immunized by intravenous injection of $1\times10^5$ SRBC and spergualin was intraperitoneally (ip) given once a day for three days starting on one day after the immunization. Four days after the immunization, mice were challenged by subcutaneous injection, of $1\times10^8$ SRBC into the footpad. Twenty four hours later footpad thickness was measured with a caliper.

* $P<0.01$.

Table 1. Effect of spergualin on mouse lymphocyte blastogenesis.

| Spergualin ($\mu$g/ml) | [$^3$H]TdR incorporation (dpm/well) | | |
|---|---|---|---|
| | Without stimulator | With Con A | With LPS |
| 0 | 284±174 | 283,681± 2,943 | 94,341±5,415 |
| 5 | 289±101 | 374,677±12,025 | 32,902±7,507* |
| 10 | 300±169 | 256,628±27,152 | 1,583±1,341* |
| 20 | 383±195 | 14,733±10,881* | 254± 133* |
| 40 | 294±169 | 519± 284* | 252± 96* |
| 80 | 230± 91 | 801± 516* | 260± 64* |

Spleen cells from CDF$_1$ mice (12 weeks old, male) are suspended at $3\times10^5$ cells/ml in RPMI 1640 medium containing 10% fetal calf serum, 100 $\mu$g/ml of streptomycin and 100 $\mu$g/ml of penicillin, and cultured with or without lectins for 3 days in 5% $CO_2$ in air. Spergualin was added at the start of the culture.

* $P<0.01$.

Table 3. Effect of spergualin on generation of alloreactive cytotoxic T lymphocyte.

| Spergualin (mg/kg/day) | Administration schedule (day) | Released $^{51}Cr$ (%) E/T ratio | | |
|---|---|---|---|---|
| | | 5 | 10 | 20 |
| 0 | | 34 | 48 | 68 |
| 5 | 1~9 | 6 | 8 | 17 |
| 50 | 1~9 | 0 | 0 | 1 |
| 50 | 9 | 21 | 30 | 51 |

P815 mastocytoma (H-$2^d$, $3\times10^7$ cells) was ip injected to C57BL/6 mice (10 weeks old) on day 0. Twelve days after the injection, spleen cells were taken from the mice and the alloreactive cytotoxic T lymphocyte activity against $^{51}Cr$-labeled P815 mastocytoma cells as the target cells was assayed.
* E/T: Effector/Target.

Table 4. Inhibition of rat skin graft rejection by spergualin.

| Spergualin (mg/kg/day) | Rat (No.) | Days before rejection (days±SD) |
|---|---|---|
| 0 | 9 | 8±2 |
| 50 | 12 | 17±3* |

SHR rats and F344 rats (9 weeks old) were used. The tail skin ($5\times10$ mm each) of SHR rats was transplanted to F344 rats. Spergualin was given intraperitoneally to the F344 rats once a day starting on one day after the grafting.
* $P<0.01$.

rejected 8±2 days after the transplantation (Table 4). Spergualin prolonged the transplanted duration up to 17±3 days when 50 mg/kg/day was given intraperitoneally for 10 days starting on one day after the transplantation.

As described above, spergualin inhibits skin graft rejection and lacks bone marrow toxicity. This suggests that spergualin or its analogues may have potential usefulness in organ transplantation.

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